Genetic imprinting: **Silencing elements have their say**

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Tissue-specific silencing elements have been identified that are required for imprinting of the individual genes in the *Igf2–H19* domain of the mouse genome. These elements further elaborate the differences between the two parental chromosomes, and add a new feature to parent-of-origin-specific gene regulatory complexes.

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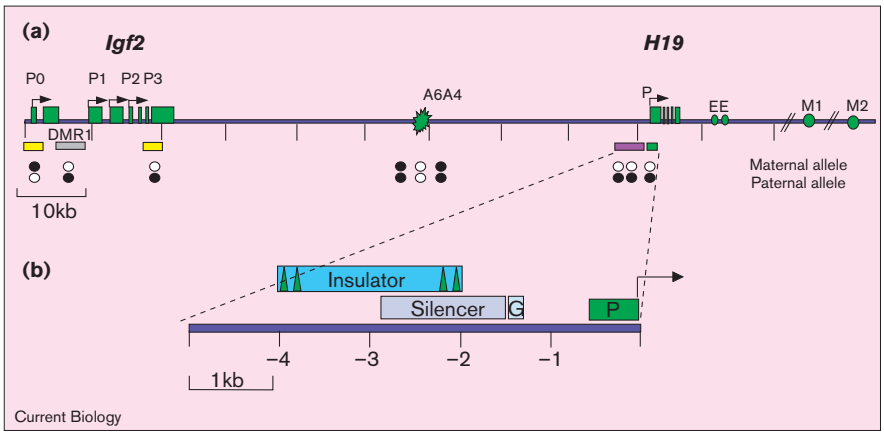
In the mouse genome, the *Igf2* and *H19* genes lie within a 120 kilobase domain at one end of a 1 megabase region containing approximately twelve imprinted genes. The two genes are separated by 90 kilobases and are oppositely imprinted, with the *Igf2* gene being expressed from the paternally inherited allele and *H19* being active on the maternally inherited copy. During development, the two genes are expressed in the same tissues monoallelically, except in parts of the brain where *Igf2* is biallelically expressed. Early studies showed that, during development, the two genes use common regulatory elements both for their expression and their imprinting, and over the past few years, a model has been evolving to explain how the allele-specific differences in transcription between the maternally and paternally inherited chromosomes are regulated. Recent studies have identified tissue-specific silencing elements that are involved only in the imprinting of individual genes.

Differentially methylated regions

Imprinted gene activity or repression at the *Igf2–H19* locus is regulated by modifications to DNA and chromatin. Chromatin structure and modification associated with imprinting have been reviewed elsewhere [1,2]. Embryos that are deficient for a methylating enzyme, the DNA methyltransferase Dnmt1, show biallelic *H19* expression and no *Igf2* activity, indicating that DNA modification by methylation is a positive regulator of *Igf2* and a negative regulator of *H19*. Local sites with DNA methylation patterns that differ between the two parental chromosomes have been identified in several studies, and these differentially methylated regions are thus implicated in the control of imprinted gene expression. These DNA methylation differences include the germline imprints that are imposed during gametogenesis, and that are considered to be critical for setting up heritable allele-specific imprints after fertilisation (reviewed in [3]). The locations of the four different types of differentially methylated region that have been identified in the *Igf2–H19* locus are illustrated in Figure 1a.

Other parts of the *Igf2–H19* locus have been identified that are not differentially methylated regions, but which nevertheless act as regulators. These include a conserved

Figure 1



(a) A schematic representation of the *Igf2–H19* locus. Four different types of differentially methylated region have been identified in the *Igf2–H19* domain, and these are indicated by different coloured bars: purple bar, germline methylation mark upstream of *H19* [13]; green bar, differentially methylated region of the *H19* promoter, which becomes fully methylated post-fertilisation on the paternal allele and is unmethylated on the maternal allele in all tissues [14]; yellow bars, two differentially methylated regions of *Igf2* which are differentially methylated post-zygotically in a tissue-specific manner [15]; grey bar, DMR1 of *Igf2*, which is partially methylated on both alleles but to different extents on each chromosome and more in mesoderm than in endoderm [9,16]. For each region, the methylated parental allele is shown as a filled circle beneath the region (upper circle maternal, lower circle paternal). Green boxes represent exons, and arrows indicate direction of transcription. P, promoter; EE, endoderm enhancers; M1 and M2, putative locations of mesoderm-specific enhancers; A6A4, conserved intergenic region. **(b)** An enlarged representation of the *H19* upstream region. The triangles represent the putative consensus CTCF-binding sites associated with insulator function. G box, repeats.

unmethylated intergenic region (see below) that is embedded in methylated DNA (A6A4 in Figure 1a) and two endoderm-specific enhancers located downstream of *H19* that act on both genes (Figure 1). The two genes are also strongly expressed in mesodermal tissues. The mesoderm-specific enhancers have not been identified, although several studies, using different approaches, have mapped their general locations to at least two separate positions, downstream of the *H19* endodermal enhancers [4–6]. It is assumed that the mesodermal enhancers are also shared by the two genes.

The insulator model

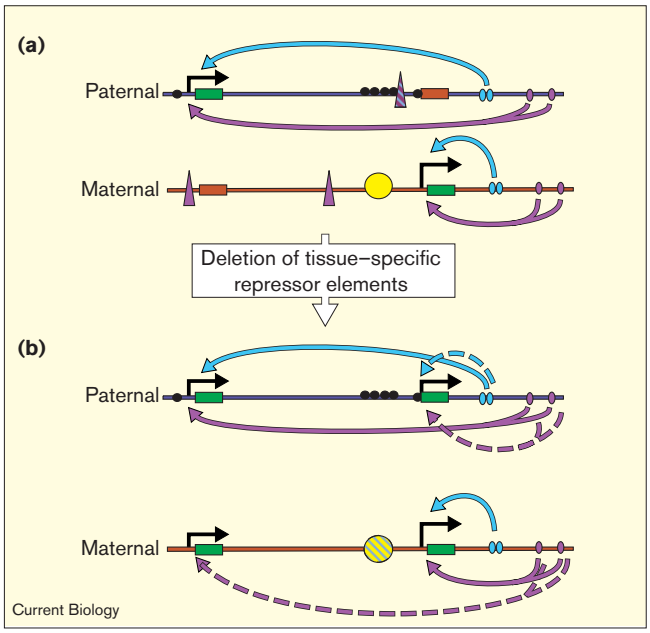
Much attention has focused on the differentially methylated region upstream of *H19*, as this carries a germline imprint (purple bar in Figure 1a). This differentially methylated region contains a number of discrete sequence elements, including an insulator element which is capable of binding the zinc-finger protein CTCF in a methylation-dependent manner, a region which can act as a silencer element in *Drosophila* and a G-rich repeat element whose function, if any, is not known (Figure 1b). Deletion of the *H19* differentially methylated region results in loss of imprinting; in general, *H19* is activated on paternal inheritance of the deletion, with a reduction in *Igf2* expression, and *Igf2* is activated upon maternal inheritance of the deletion, with a reduction in *H19* expression [7].

The current model to explain the monoallelic expression of *H19* from the maternal chromosome and *Igf2* from the paternal chromosome is a simple one, if the two parental chromosomes are considered seperately (Figure 2a). On the maternal chromosome, the key players are the unmethylated, position-dependent insulator element located upstream of *H19*, the unmethylated *H19* promoter and the aforementioned enhancers. The unmethylated insulator sequence can bind the zinc-finger binding protein CTCF. This insulates the unmethylated *Igf2* promoter from the downstream enhancers and allows transcription from the unmethylated maternal *H19* promoter (Figure 2a). On the paternal chromosome, the upstream differentially methylated region is initially required for the *H19* promoter methylation that is involved in preventing the enhancers from driving *H19* transcription [8]. So what drives the activity of the active *Igf2* allele on the paternal chromosome? Several studies have shown that the insulator, which is methylated on the paternal allele, can no longer bind CTCF; hence the enhancers are no longer insulated from *Igf2*, allowing the paternal allele to be transcribed (Figure 2a; reviewed in [2]).

Modulating insulation

These studies implicate the differentially methylated insulator upstream of *H19* as the key regulator of domain imprinting, and suggest that the *Igf2* locus has little influence on regional control. But three recent papers

Figure 2



(a) Schematic representation of the *Igf2–H19* domain on the two parental chromosomes, with the newly identified silencer elements indicated as triangles. Mesodermal functions are indicated in purple and endodermal functions in pale blue. The CTCF bound insulator is shown as a yellow circle. Methylated regions are shown as black circles, and enhancers as ovals downstream of *H19*. Coloured arrows illustrate tissue-specific enhancers driving transcription from the promoters. **(b)** Deletion of the tissue-specific silencers (triangles) affects each gene independently of the other. The original enhancer functions do not appear to be affected by the deletions; however, additional activation is now evident, as shown by the dotted arrows. Endodermal functions are represented in pale blue and mesodermal in purple. The insulator function appears to be compromised in mesodermal tissues on the maternal chromosome. The diagram represents a summary of the three *separate* deletions and is not intended to suggest that deleting all three at once will necessarily result in the illustrated outcome. (Drawing not drawn to scale.)

[9–11] have added a new level of complexity to this simple insulator model. These papers report the identification of tissue-specific, *cis*-acting regulators of imprinting; specifically these regulators function on one of the two parental chromosomes and confer tissue-specific repression. Furthermore, these elements are gene-specific repressors.

It had been proposed that unmethylated differentially methylated region 1 (DMR1; grey bar in Figure 1a) on the inactive *Igf2* allele is able to bind a repressor which is associated with silencing of this allele. The insulator model, however, suggests that repression of *Igf2* is regulated by the differentially methylated region upstream of *H19*. Recently, Constancia *et al.* [9] have deleted the *Igf2* DMR1 to address whether it has any role to play in imprinting. While paternal transmission of the deleted (normally methylated) DMR1 has no effect on fetal *Igf2* transcription, maternal transmission of the deletion *Igf2* DMR1

results in loss of imprinting of *Igf2*, but only in several mesodermal tissues (Figure 2b). This was manifested as biallelic expression, though the usually silent maternal *Igf2* allele was not activated to the levels normally transcribed from the active paternal allele. There was no effect on the expression of either allele of *H19*. One likely explanation for these data, suggested by Constancia *et al.* [9], is that the mesoderm enhancers are not fully insulated from the inactive *Igf2* allele, and require additional elements, such as the unmethylated *Igf2* DMR1, for mesoderm silencing.

The need for additional elements for tissue-specific insulation is further substantiated in an earlier report by Ainscough *et al.* [10], in which a small conserved part of the intergenic A6A4 region was deleted (Figure 1). Again, loss of *Igf2* imprinting on the maternal chromosome was observed in a mesoderm derivative, again in the absence of an effect on *H19*. The resulting biallelic *Igf2* expression was highly tissue-specific, being confined to skeletal muscle. As in the work of Constancia *et al.* [9], the paternal chromosome was apparently unaffected in fetal tissues. Two groups [9,10] have therefore identified distinct, mesodermal-specific silencer elements which can, at least partially, dissociate repression of the maternal *Igf2* allele from the regulatory elements around *H19* in mesodermally derived tissues (Figure 2b).

From these studies there is still no proof that differential methylation at *Igf2* is important for tissue-specific imprinted repression. In the work of Ainscough *et al.* [10], the deleted sequence was not a differentially methylated region, even though the deletion was found to have parent-of-origin-specific effects [10]. In the work of Constancia *et al.* [9], however, the tissues in which *Igf2* was seen to be de-repressed are those that exhibit hypomethylation. Furthermore, the authors cite unpublished work suggesting that repression can be alleviated by methylation [9]. In this case, therefore, there is a suggestion that DMR1 has methylation-dependent repressor function.

The third study, by Drewell *et al.* [11], indicates that tissue-specific repressor elements in the region are not confined to *Igf2*, to the mesoderm or to the maternal chromosome. Deletion of the *H19* upstream silencer element, a region that includes the 3′ end of the insulator locus (Figure 1b), results in retention of appropriate *Igf2* imprinting and expression, but activation of paternal *H19* expression in many tissues of both endodermal and mesodermal origin. The deletion does not affect the maternal chromosome, and hence the insulator can function on this chromosome in the absence of these sequences. Interestingly, methylation imprints were unaffected in this mutant, even on the activated paternal *H19* allele.

Although the precise nature of insulator function is not known, these studies suggest that regulatory elements exist that can fine-tune its activity. The regulatory interactions are different in different tissues, and this emphasises the need to analyse *Igf2* and *H19* expression in several tissues when characterising mutations in the domain. Now that tissue-specific elements have been identified which are potentially involved in the interaction between enhancers and insulators, and which affect promoter function, one further challenge is to determine how these interactions affect the chromatin milieu of the domain.

The insulator appears to delineate a boundary, downstream of which are enhancers associated with imprinted expression of the *Igf2* and *H19* genes. In the brain, where expression of *Igf2* is biallelic, the relevant enhancers are located upstream of this boundary [12]. The tissue-specific repressors acting on maternal *Igf2* are upstream of the boundary, while those affecting paternal *H19* are downstream. It is therefore reasonable to assume that the position of these regulators relative to each other is important. A more detailed image is now emerging, in which the maternally and paternally inherited chromosomes look strikingly different from each other with respect to the complex *cis*-acting regulatory interactions that are occurring. It will be of interest to determine the extent to which this *Igf2–H19* paradigm can be extended to other imprinted loci, and to consider what this might tell us about the evolution of regulation at imprinted domains.

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